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TECHNICAL NOTE

A new method of inducing experimental chronic renal failure by cryosurgery

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Several laboratory models of chronic renal failure have been described, namely, diminution of renal mass by surgery [1–3] or ligation of renal artery branch [4], toxic nephritis [5–7] and immunologically induced nephritis [8–10]. Each has special applicability in studying certain aspects of chronic renal disease. Most of the studies in advanced chronic renal failure have used the surgical resection of renal parenchyma to reduce the kidney mass, because immunological and toxic experimental models seldom represent the stable and far advanced renal failure [5, 6, 9]. Reduction by surgery, however, may have a disadvantage in having a risk of hemorrhage [11]. This paper describes a new and simple technique to induce reproducible chronic renal failure in rats using cryosurgery.

Methods

Adult male Wister rats aged 11 to 12 weeks and weighing 300 to 350 grams were used. Ninety-two rats were alloted for the experiment of chronic renal failure and 18 for sham operation as control. Three to five days after baseline blood and urinary biochemical studies were done, the rats were anesthetized with pentobarbital (3 mg/100 g body wt intraperitoneally). The left kidney was exposed and decapsulated through a flank incision.

Renal failure was achieved by freezing the renal parenchyma using cryosurgery (Fig. 1). The frozen cupper stick (1.2 × 0.8 × 15 cm), which was immersed in liquid nitrogen, was placed on the upper and lower poles as well as on the anterior and posterior lateral aspects of the kidney for 20 to 60 seconds, depending on the desired degree of uremia. The cooling tool did not injure the entire surface of the kidney to keep a small intact area, as shown in Figure 1. Three graded uremic rat groups (mild, moderate, severe) were made by varing the freezing time. The frozen stick was placed on four portions, 20 seconds per direction in the mildly uremic group, 30 seconds in the moderate, and 45 to 60 seconds in the severe group. Hemorrhage, a common post-operative complication, was not a problem. Two weeks after cryosurgery, the contralateral intact kidney was removed under anesthesia with ether. The sham operated animals were prepared by exposing and isolating the left and right kidney respectively in a two–stage operation.

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The rats were housed in individual metabolic cages throughout most of the experiment. The animals were given CE-2 (Clea Japan, Inc.) containing 24.5% protein and 0.85% inorganic phosphate and water ad libitum. Body wt and urinary volume were checked twice a week. Blood urea nitrogen and serum creatinine was measured every two to four weeks thereafter. Twenty–four hour endogenous creatinine clearance and other blood chemistry were determined every four weeks. Venous blood samples were obtained by orbital sinus puncture under light ether anesthesia. Urea nitrogen was determined with spectrophotometric method using a commercially available kit (65-UV, Sigma Co., St. Louis, Missouri, USA). Serum and urine creatinine was measured with Beckman Creatinine Analyzer 2 (Beckman Instruments, Fullerton, California, USA) and other blood chemistries were analyzed with Technicon autoanalyzer (SMAC, Technicon Instruments Inc., Tarrytown, New York, USA). The microanalysis of blood gas and pH was done on a Radiometer–Eschmeiler combination unit. The systolic blood pressure was measured by the tail cuff method of Friedman and Freed [12]. Statistical analysis was done by unpaired *t*-test, and a P value less than 0.05 was taken as a statistical significance.

Results

Survival rate of the animals

All rats tolerated the two–stage operation. The earliest mortality occurred in the severely uremic group. The more severe the uremia, the shorter the survival period (Fig. 2). Fifty percent of the population died within 15 weeks in the mildly uremic group, 11 weeks in the moderate, and 5.5 weeks in the severe group. All rats in the severely uremic group died within 12 weeks. The majority of rats manifested weakness, malaise, seizure, and diarrhea attributable to uremia.

Body weight

In this experiment, body served as a good indicator of how uremic the rat was. The degree of uremia paralleled the wt curves of the rats. The severe group had significant wt loss, the moderate did not gain or lose wt, and the mild group had wt gain which was significantly less than sham operated control (Fig. 3). A significant reduction in food consumption was noticed in the severe and moderate uremic groups compared with sham (data are not shown).

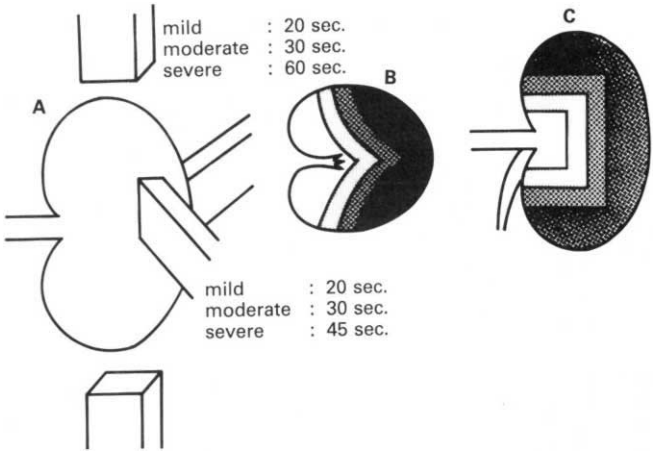

Fig. 1. *A. Method of cryosurgery.* The frozen cupper stick was placed on the upper and lower portions of the kidney for 20 to 60 seconds and on the lateral portions for 20 to 45 seconds depending on the degree of uremia. **B.** Horizontal section of the injured kidney. The shaded area represents the degree of injured parenchyma, (■) mild cryosurgery, (▨) moderate cryosurgery, ▩ severe cryosurgery, □ intact parenchyma.

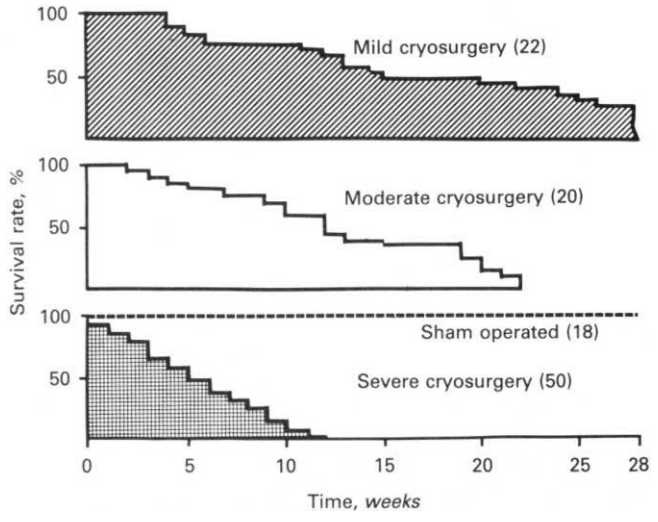

Fig. 2. *Survival rate of rats with three graded uremia.* Numbers in brackets indicate number of rats.

Urine volume

Urine volume was significantly greater in uremics than sham control after contralateral nephrectomy throughout the experiment. The more severe the renal failure, the greater the daily urinary volume. The mean urine volume was 13 ± 2.6 ml in control, 25 ± 9.7 in the mild, 39 ± 12.3 in the moderate and 47 ± 12.3 in the severe uremics.

Serum creatinine

Serum creatinine (S-CRTN) and blood urea nitrogen (BUN) rose transiently during the first week following nephrectomy. During the second week, S-CRTN and BUN dropped to some extent. Thereafter, S-CRTN and BUN progressively increased, with the curve of the severe group having the steepest slope at the earliest time. The level of S-CRTN started to increase on the fourth week in the severe group, eighth week in the moderate, and 20th week in the mild group (Fig. 4).

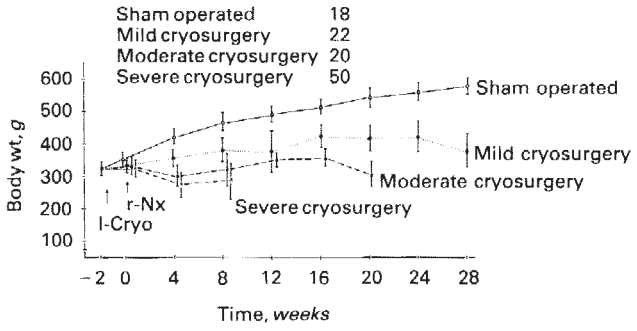

Fig. 3. *Change of body wt after induction of chronic renal failure.* Values are the mean ± 1 standard deviations.

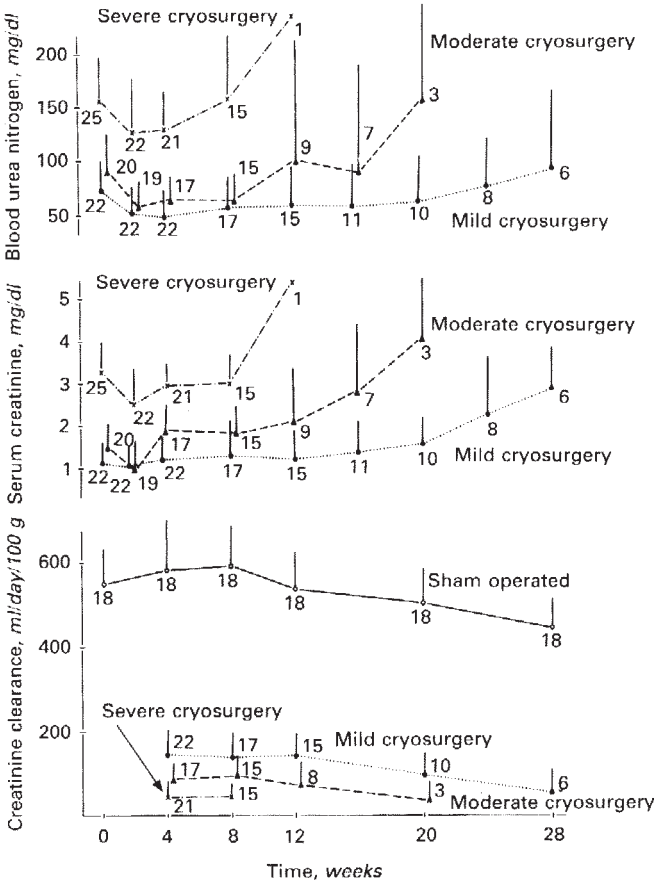

Fig. 4. *Changes of blood urea nitrogen, serum creatinine and 24 hour creatinine clearance.* Number of each point represents the number of studied animals.

Twenty–four hour creatinine clearance

Twenty–four hour creatinine clearance (Ccr) of control was 590, 74.5 ml/day per 100 g body wt. Ccr of the mild, moderate and severe groups were 21% (122 ± 37.5), 15% (87 ± 11.7), 8% (45 ± 8.9) of the sham control respectively (Fig. 4).

Table 1. Systolic blood pressure (S.B.P.), blood gas, hematocrit (Ht), serum calcium and phosphate concentration in the control and in the severely uremic rats eight weeks following nephrectomy.[a]

	S.B.P. (*mm Hg*)	pH	HCO_3 (*mEq/liter*)	Ht (%)	Ca (*mg/dl*)	PO_4 (*mg/dl*)
Control	128	7.403	24.6	48.3	9.7	7.6
(N = 12)	± 9	± 0.007	± 0.9	± 3.0	± 0.56	± 1.28
Uremics	148*	7.302*	19.8*	36.5*	8.3*	14.8*
(N = 13)	± 32	± 0.022	± 3.3	± 10.0	± 0.76	± 11.51

[a] Blood gas and other blood chemistry were measured simultaneously in the blood drawn from orbital sinus. Data are the means ± SD. Asterisk means significant difference from control.

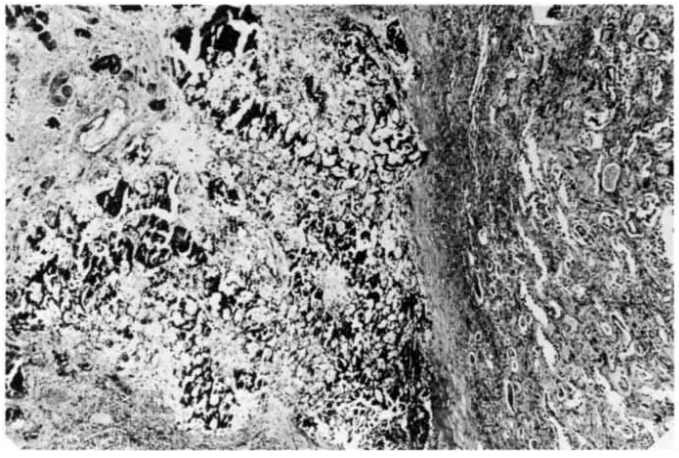

Fig. 5. *Almost complete cicatrization with marked calcification of the injured area (right two of the third) separated by fibrotic zone, with small round cell infiltration from the nonaffected area (H & E stain, ×17).*

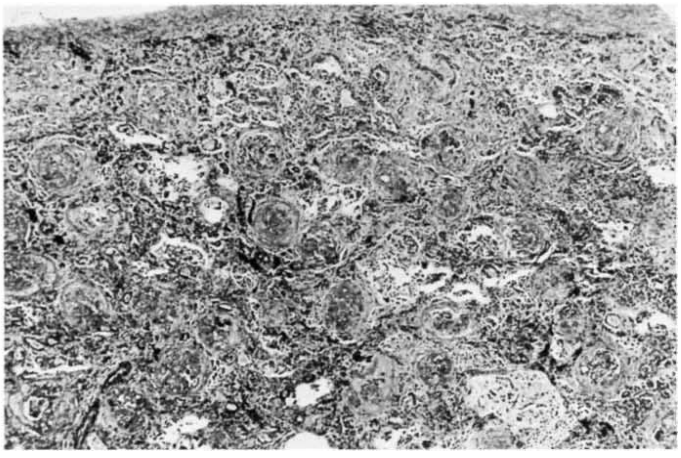

Fig. 6. *The cortex of the injured kidney showed evident glomerular scar and cicatrical mesangial proliferation, and marked small round cell infiltration in the interstitium with slight fibrosis (H & E stain, ×83).*

Systolic blood pressure and other parameters

Table 1 shows the systolic blood pressure of the tail artery, blood gas, hematocrit and blood chemistry in the control and severely uremic rats eight weeks following nephrectomy. Hypertension, metabolic acidosis, anemia, high serum phosphate, and low calcium concentration were noticed among the uremic rats.

Histopathological findings

On light microscopy, the sections from injured renal tissue within two weeks after cryosurgery showed widespread acute renal infarction. Four to six weeks later, the injured area fell into cicatrization with marked calcification separated by fibrotic zone from the nonaffected area (Fig. 5). The interesting findings were the glomerular lesions in the later stage, which showed marked hyalinization and sclerotic mesangial proliferation with marked interstitial small round cell infiltration and slight fibrosis, such as chronic glomerulonephritis in end stages (Fig. 6).

Discussion

Reduction of renal mass by surgical resection of renal parenchyma or by ligation of renal artery branches seem to be the most commonly used technique in experimental uremia. The former sometimes encounters problems in hemostasis of dissected parenchyma or difficulties in archieving far-advanced renal failure. Damage to renal calyces which results in urinary leakage or perirenal abscess may complicate the procedure. The latter cannot always obtain reproducibility in terms of the severity of induced uremia due to differences in arterial supply. Several methods have reported to result in a more profound uremia without the risk of hemorrhage [11, 13–15]. Boudet et al [11] described an interesting method using a specially devised electrocoagulation which induced progressive uremia over several months. The disadvantages are the requirement of special equipment and the difficulty in predicting at the time of operation how uremic the rats will be.

Our method offers distinct advantages. Unlike other techniques, our procedure does not entail the risk of hemorrhage. Because there was no extra effort to exert hemostatic control, it makes our method simpler. It can induce a stable and reproducible model of chronic renal failure. The desired degree of uremia can be produced by controlling the freezing time. Post-operative mortality is nil. Contralateral nephrectomy should be done at least two weeks after initial cryosurgery to allow the remnant kidney to recover from swelling, and thereby reduce the mortality rate in the immediate postnephrectomy period. Acetone and dry ice can also be used instead of liquid nitrogen, however, they are more expensive.

Although this new model is similar to other surgical methods to reduce nephron mass, immunological mechanisms might be involved to some extent in the progression of renal injury with this procedure. Several investigators have observed autoanti-

body formation after cryosurgery [16, 17]. Kanetake [18] showed that circulating antibodies against renal extracts were present in the serum of rabbits after renal cryosurgery, and IgG deposits were found along the glomerular basement membrane and in the mesangium.

The plasma level of angiotensin I was not different between sham rats and rats with cryosurgically-induced uremia [19], Additional pathophysiological analyses are necessary to fully understand this type of uremia.

The mean serum creatinine (S-CRTN) was not appreciably elevated in the mildly uremic rats. However, half of them had S-CRTN values of more than 2.5 mg/dl; these animals died by the 16th week of the experiment. S-CRTN just before death seemed to be lower in the mildly uremic group compared to the other two groups. Higher protein intake could be a possible explanation. According to our recent study, uremic rats given a high protein diet died earlier with significantly lower S-CRTN than those given a normal protein diet [20].

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